Microfluidics for 3D dynamic cell cultures in 384 multiwell format

P. De Stefano[1], G. Venturini[1], O. Botrugno[2,3], G. Tonon[2,3], G. Dubini[1] and E. Bianchi[1]
[1] *Laboratory of Biological Structure Mechanics (LaBS), Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Politecnico di Milano, Milan, Italy*
2. Functional Genomics of Cancer Unit, Division of Experimental Oncology, IRCCS San Raffaele Scientific Institute, Milan, Italy
3. Università Vita-Salute San Raffaele, Milan, Italy

***Abstract*— Drug development is a lengthy and costly process, characterized by a high failure rate due to unreliable pre-clinical models, such as 2D cell cultures, which fail to replicate complex biological systems. 3D cell cultures and organ-on-a-chip technology offer more accurate representations of human tissues, replicating key factors like cell interactions and blood flow. Miniaturization and standardization are key for advancing these technologies. Combining 3D printing with Polydimethylsiloxane (PDMS) enables the creation of custom-designed prototypes that enhance the precision, scalability, and reproducibility of these advanced models.**

Here, we present a proof of concept of a well prototype, 384 multiwell format, constituted by an inlet connected to a syringe pump and an outlet, made of two components: an 8 mm 31 G needle and a 3D printed PLA dropper.

The prototype was investigated by studying droplets volume, the relation between the 'dripping rate' and 'inlet flow rate' and the maximum sustainable flow rate. Three different fluids were used for these analyses: distilled water, phosphate buffer solution (PBS) and cell culture medium.

The results showed that all three droppers exhibited similar behaviour across different fluids. At flow rates above 1 µL/min, the dripping time increased as the flow rate decreased, as expected. However, at lower flow rates, the dripping time was nearly twice as long due to flow inertia, causing a delay in droplet formation. The operational range of the droppers varied, with the 1.5 mm and 2 mm droppers showing a narrower range compared to the 2.5 mm dropper, especially when using cell culture medium. This suggested a correlation between the dropper's performance and the type of fluid used.

***Keywords* — Microfluidics, 3D printing, drug screening, miniaturization**

I. Introduction

DRUG development is a lengthy and costly process, with a high failure rate due to the use of unreliable pre-clinical models that fail to accurately replicate complex biological systems [1], [2]. High-Throughput Screening (HTS) systems, commonly used to simultaneously test large compound libraries, lack in predicting outcomes because they rely on 2D cell cultures and static conditions [3], [4]. This approach is too simple and does not account for critical factors such as cell-to-cell interactions, tissue architecture, or the influence of the extracellular matrix, resulting in unreliable data [3].

In contrast, 3D cell cultures, such as patient-derived organoids (PDOs), represent a promising alternative, offering a more accurate representation of human tissues. Organoids are self-aggregating three-dimensional structures that closely mimic the architecture and functionality of organs, providing more physiologically relevant conditions [4].

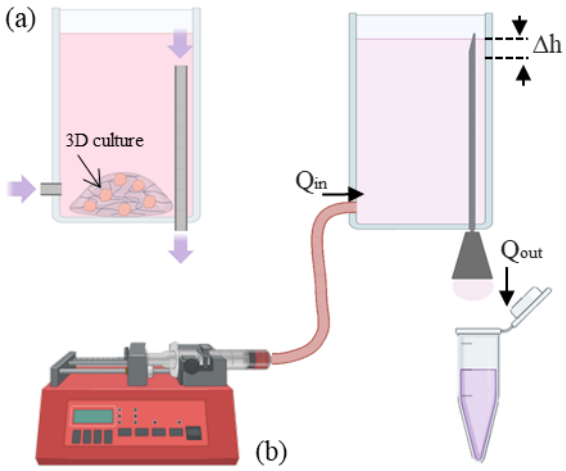

Fig. 1: Schematic representation of the system. (a) Example of the well containing 3D cell culture, having a horizontal inlet and a vertical outlet, made of a 31G needle and a 3D printed dropper. Δh defines the fluid level variation which determines the volume of the outlet droplet. (b) Experimental setup with syringe pump administering fluid to the well and a 1.5 mL tube for eluate collection. Created in BioRender. Bianchi, E. (2025) https://BioRender.com/0a0wazw.

Organ-on-a-chip technology advances this concept by using microfluidic devices to simulate the mechanical and biochemical characteristics of human organs. These chips create dynamic, flowing environments that better replicate blood flow and the effects of nutrients, oxygen, and waste products on tumour cells [5], [6]. Miniaturization of 3D cell cultures is key to increase the throughput of the analyses, advancing the output and the research impact. However, it increases the complexity of the systems, hampering their usability, and often lacks standardization. The use of 3D printing technology can help in overcoming this problem, enabling precise fabrication of complex microstructures at the millimetre scale, and the creation of custom-designed chips with high accuracy.

The possibility to create a custom 384-well plate system, where each well is equipped with an inlet and outlet, can enable independent and dynamic culture conditions for PDOs. This setup would support High-Throughput Screening assays under more physiologically relevant, flow conditions offering a significant step forward compared to traditional static systems. By combining polydimethylsiloxane (PDMS) with 3D printing, we have developed a well prototype (384 multiwell format) that serves as an initial proof of concept of

a High-Throughput system for in-flow drug screening on PDOs.

II. MATERIALS AND METHODS

A. Design and fabrication of prototype

The system is constituted by a syringe pump (NE-300, New Era Pump Systems Inc., Farmingdale, New York, United States), a PDMS well and a 1.5 mL tube (fig.1b). A PTFE

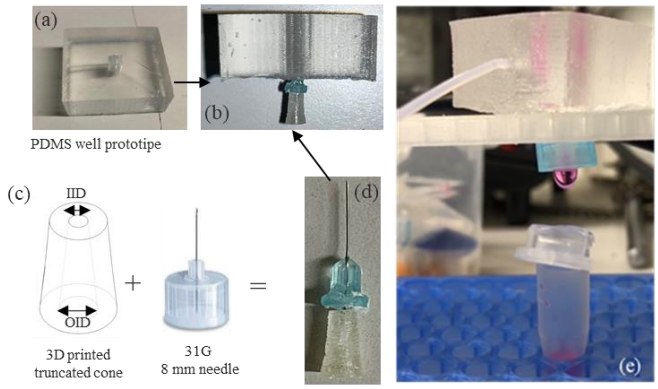

Fig.2 Pictures of the well. (a) PDMS well prototype, (c) single components and (d) assembled outlet, (b) assembled system. (e) Working Prototype during a test.

tubing (0.5 mm ID, 1.6 mm OD) connects the pump to the fluidic inlet of the well, located on its sidewall.

The well, open on the top, has an outlet through the bottom side and it is made of two components: a commercial 8 mm 31 G needle (PIC Insupen Original) and a 3D printed dropper (Fig.2a). The well diameter is approximately 3.45 mm as the one of a standard 384 multiwell plate. The PDMS well was produced using a 3D printed mold, designed in SolidWorks (Dassault Systèmes, France), and printed with an FDM 3D printer (Fused Deposition Modelling Verve, Kentstrapper Srl, Florence, Italy) in polylactic acid (PLA). A 23 G syringe needle was inserted into the 3D-printed mold to create the inlet channel. Liquid PDMS (Sylgard 184 Silicone Elastomer Kit), mixed at a ratio of 9:1, was poured into the mold and cured at 90 °C for 15 minutes. Afterwards, the needle was removed and PDMS prototype detached.

The 3D printed dropper, designed as a truncated cone was produced with the same technology as the well mold. It was designed using SolidWorks and printed with FDM 3D printer. To investigate the influence of the dropper dimensions on the outlet droplet formation and dripping, three droppers with different Outlet Internal Diameters, OID, (1.5, 2, 2.5 mm) were produced. The Inlet Internal Diameter, IID, was, instead, kept constant and equal to 0.5 mm. Liquid flows into the well through the inlet and is then captured by the outlet needle that is close to the free surface of the fluid inside the well. Fluid flows through the needle to the truncated cone, giving rise to a drop that grows till the final detachment and falls into the tube.

B. Prototype testing

The prototype was characterized by studying the relation between the dripping rate (DR), the inlet flow rate (Q_{IN}), and the maximum sustainable flow rate Q_{MAX}. Increasing Q_{IN} values (1, 5, 10, 20, and 25 µL/min) were applied at the inlet of the well. Dripping time (t_D), which corresponds to the time between the detachments of two consecutive droplets, was measured with a timer. Maximum sustainable flow rate Q_{MAX} was, instead, evaluated by defining the value at which the fluid level increases inside the well, above the top of the outlet. To analyse the dropper behaviour under different settings, three different fluids at room temperature were tested: distilled water, phosphate buffer solution (PBS), and cell culture medium. Tests were performed in triplicates.

To determine droplets' volume, 10 droplets were collected in a 1.5 mL tube, which was weighted before and after the test. This procedure was repeated three times for each dropper.

III. RESULTS AND DISCUSSION

A dripping system was designed to collect media from the outlet of each well, passively facilitating and controlling the eluates collection. The system consists of a vertical channel that sets the maximum height of the media within the well, and a dropper, designed as a truncated cone with an internal diameter that progressively increases toward the outlet (fig. 2).

To optimize the dripping process, three different droppers with increasing OID (1.5, 2, and 2.5 mm) were 3D printed and tested. One test focused on determining droplet volume for each dropper size and fluid type. As shown in fig. 3, the volume of each droplet was influenced by both the size of the dropper and the type of fluid. As expected, a larger dropper diameter resulted in a larger droplet volume. This is because the critical mass required for droplet detachment is proportional to the radius of the dropper. Droplet volume also depends on the fluid's surface tension, which varies according to its composition. For example, droplets formed from PBS were statistically significantly larger, as the dissolved salts in the solution increase the surface tension by promoting stronger intermolecular bonds between water molecules and salt ions. In contrast, distilled water, which lacks salts and impurities, has a lower surface tension (72 mN/m), resulting in smaller droplets. Cell culture medium, with a surface tension of 44-54 mN/m [7], formed even smaller droplets compared to both PBS and distilled water, as seen in the third graph (fig. 3).

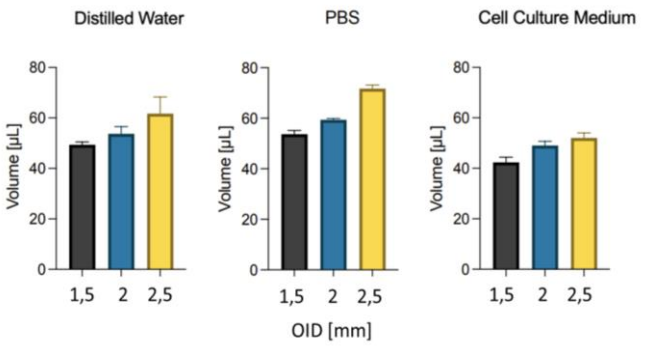

Fig. 3 Graphs reporting the mean value of droplets volume obtained using three different OID (1.5, 2 and 2.5 mm) and three different fluids (distilled water, PBS and cell culture medium).

In addition to droplet volume, flow rates and dripping time (the interval, t_D, between the formation and detachment of two

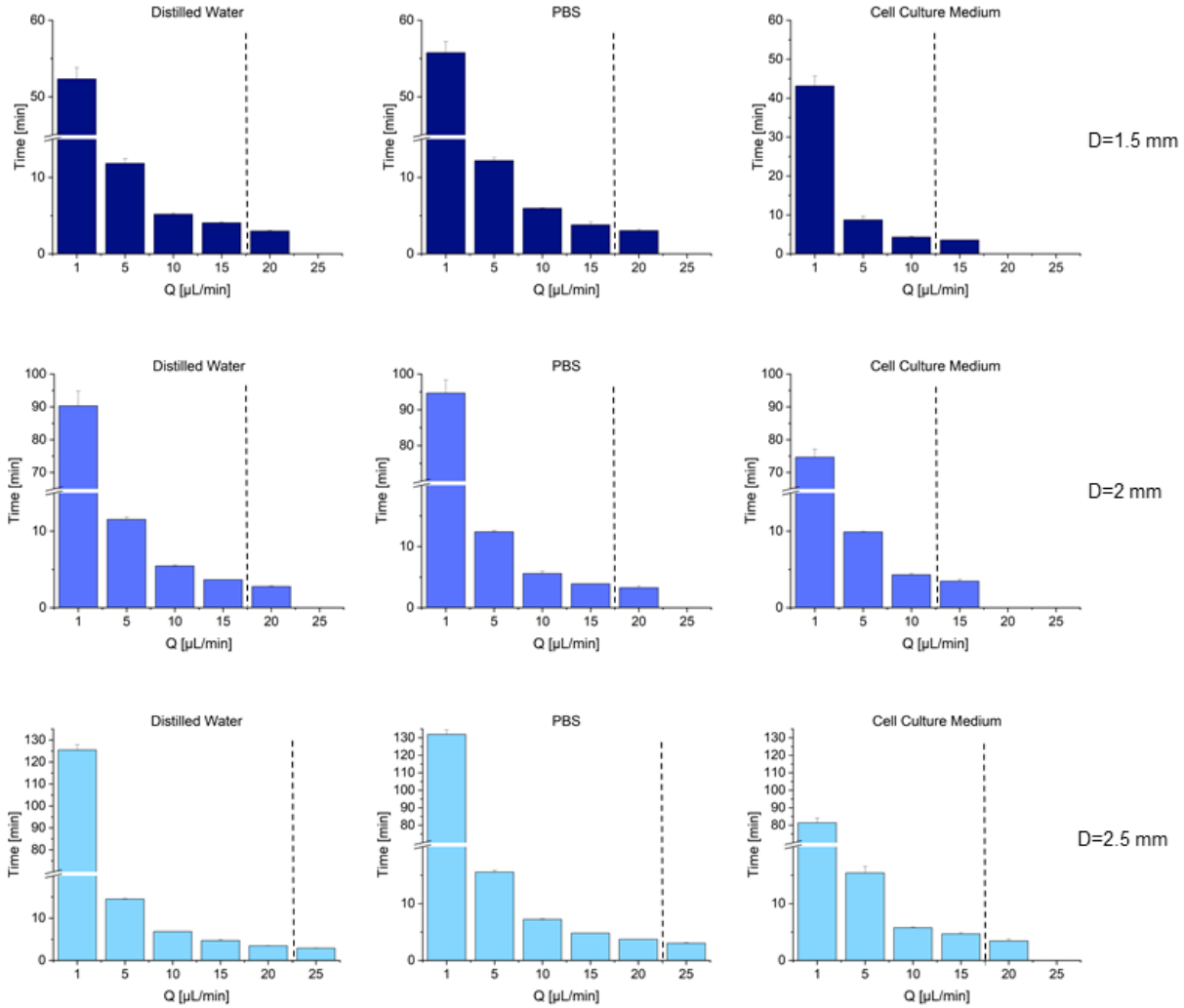

Fig. 4 – Graphs reporting the dripping time of the three droppers configurations, having variable OID (1.5, 2, 2.5 mm) with the three different fluids (distilled water, PBS and cell culture medium). The dashed line in each graph separates the flow rate conditions the droppers were able to sustain (on the left) from the ones that resulted in a progressive filling of the well (on the right).

consecutive droplets) were measured using a chronometer timer in video recordings. The purpose of these tests was to determine the maximum flow rate the dripping system could accommodate. It was observed that beyond a certain flow rate limit (Q_{MAX}), the system could no longer balance the inlet flow rate, causing the well to gradually fill and overflow. The measurements were repeated three times for each condition, and tests were performed with distilled water, PBS, and cell culture medium. The results for the three droppers are shown in fig. 4.

As shown in the graphs, all droppers exhibited similar behaviour, and this trend remained consistent when switching between fluids. For flow rates above 1 µL/min, the dripping time increased as the flow rate decreased, aligning with the expected time for a droplet of a given volume to detach. However, for lower flow rates, the dripping time became prolonged, nearly twice as long as anticipated. This could be attributed to flow inertia: after a droplet detaches, fluid continues to move within the needle for a brief period, causing the meniscus to drop below the height needed to generate the required pressure drop for continued dripping. Since the flow rate is extremely low, the liquid level in the well slowly returns to its original state, causing a delay in the formation and detachment of the next droplet.

The dotted line in each graph distinguishes the flow rate conditions the droppers could sustain (on the left) from those that led to progressive well filling (on the right). Both the 1.5 mm and 2 mm droppers showed the same operational range, which was narrower than that of the 2.5 mm dropper. A reduction in the operational range was also observed when switching to cell culture medium, indicating a correlation between the dropper's ability to match the inlet flow rate and the volume of droplets formed with a specific liquid.

IV. Conclusion

In conclusion, the device prototype successfully meets the requirements of both encumbrance and functionality, demonstrating its effectiveness in the final application. However, as currently designed, it is not scalable due to both the use of PDMS and the dependence on multiple assemblies, which hamper reproducibility. To make the device more scalable and reproducible, a new fabrication process is needed. Additionally, consolidating the design into a single piece rather than multiple components would enhance

reproducibility and improve alignment, ensuring more reliable performance in large-scale applications.

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